Improving Attachments of Remotely-Deployed Dorsal Fin-Mounted Tags: Tissue Structure, Hydrodynamics, in situ Performance, and Tagged-Animal Follow-up

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LONG-TERM GOALS

We recently developed small satellite-linked telemetry tags that are anchored with small attachment darts to the dorsal fins of small- and medium-sized cetaceans. These Low Impact Minimally-Percutaneous External-electronics Transmitter (LIMPET) tags have opened up the potential to monitor the movements of numerous species not previously accessible because they were too large or difficult to capture safely, but too small for tags that implant deeply within the body. In this project we aim to improve upon our existing tagging methodology to achieve longer, less variable attachment durations by carefully examining the factors that affect attachment success. Our key goal is to develop a method for attaching tags to cetaceans that provides the data needed to answer critical conservation and management questions without an adverse effect on the tagged animal. Therefore, our project includes follow-up studies of whales that have been tagged with a remotely-deployed dorsal fin-mounted tag to accurately quantify wound healing and the effects of tagging on whale survival, reproduction, and behavior. The combination of these approaches should provide an improved understanding of some of the key factors affecting tag attachment duration as well as a more complete understanding of impacts to individuals due to tagging.

OBJECTIVES

1. Design an improved barnacle-style tag shape for remote-deployment by assessing the hydrodynamic properties of tag shapes
2. Examine the tissue structure of the dorsal fin and its material properties for better informed implanted attachment design.

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3. **3.** Examine the in situ performance of our current attachment devices and then design and test improved retention systems
4. **4.** Conduct follow-up studies of tagged whales to accurately quantify wound healing and the effects of tagging on whale survival, reproduction, and behavior

APPROACH

1. Hydrodynamics of tag shape (Key individuals: Mittal, Howle, Andrews, Schorr, Hanson): We will determine the drag on the tag through numerical modeling and analysis, primarily computational flow dynamics. High fidelity numerical simulations working in concert with physical experiments will be used to establish qualitative as well as quantitative relationships between tag configuration and the associated flow structure and surface pressure distribution, which is ultimately the key to the force/moments on the tag.

2. Dorsal fin tissue structure (Key individuals: Hanson): To evaluate the factors influencing tissue degradation (and therefore attachment duration) we will assess the anatomical factors likely to influence long-term viability. Interspecific differences in these parameters may be an important factor in the variability in attachment duration, as might body size scaling effects. We selected various species for examination based on differences such as size/shape of the dorsal fin (e.g., melon-headed versus killer whale), taxonomic grouping (i.e., odontocetes versus mysticetes), or dynamic behavior (*e.g.*, beaked whale versus melon-headed whale).

3. Performance of tag attachments – simulated and actual (Key individuals: Andrews, Schorr, Hanson, Howle, Mittal): A key factor in attachment duration is likely the hydordynamic forces imposed by the tag body but acting on the attachment elements implanted into the dorsal fin. Although we have a good idea of how the LIMPET retention system operates when first implanted, we do not fully understand the mechanics in a living fin. Therefore, we will use non-invasive imaging of carcass tissue to determine how the retention elements behave in situ. These results, along with those from the analysis of dorsal fin histology and material properties will inform modified designs. Hundreds of LIMPET tags have now been applied to various species of cetaceans, and we will examine inter-species differences and explore factors that effect tag longevity.

4. Tagging effects - follow-up studies of survival, reproduction,& behavior (Key individuals: Baird, Schorr, Andrews, Hanson): More thorough assessments of the potential impacts on survival and reproduction of individuals, as well as assessment of healing of the tag attachment sites and potential behavioral changes associated with tagging, are needed to address concerns regarding sub-lethal and potentially lethal impacts of remotely-deployed tags. As part of an ongoing collaborative study, over 130 satellite and VHF tags have been remotely-deployed on 9 species of odontocetes around the main Hawaiian Islands. Re-sighting rates for the two species with the largest sample size of tag deployments, short-finned pilot whales and insular false killer whales, are particularly high, as populations are small, individuals are relatively easy to approach, and there are sufficient encounters each year to have a high probability of re-sighting previously tagged whales. We will assess impacts of remotely-deployed tags on tagged animals at a variety of levels: from wound healing and potential behavioral effects of tag attachment to reproduction and survival. Assessment of reproduction and survival of tagged whales will utilize existing photographic datasets as well as additional photos taken during this project.

WORK COMPLETED

Objective 1:
The majority of the work on the hydrodynamics of LIMPET tags was completed in the first two years of our project. Both Computational Fluid Dynamics (CFD) studies and water tunnel tests with physical models were conducted to compare the most recent iterations of the LIMPET tag designs and potential improved designs. During this final year we worked to compile these results and synthesize our understanding of the hydrodynamics and biomechanics of the tags and implants.

Objective #2:
During the initial phases of this project, dorsal fins were collected from beach-cast specimens of eight species of cetaceans – harbor porpoise, pilot whale, killer whale, false killer whale, pygmy killer whale, Bryde's whale, Cuvier's beaked whale, and Blainville's beaked whale. We used these dorsal fins to assess the geometry and collagen composition of primary structural layers, and to determine the stress/strain characteristics of the load-bearing tissues (the ligamentous sheath and the central core). In addition to examining differences between species, we used the killer whale fin to examine the potential variation in collagen composition and material properties at various locations of the fin. Material properties of the two primary collagen-bearing layers of the dorsal fin (ligamentous sheath and central core) were measured for these 8 species on a uniaxial tester to build stress/strain curves. In order to assess the strength of the tissue of the eight cetacean species, and five regions of the killer whale fin, the yield point was estimated by fitting a hierarchical Bayesian Gompertz growth model to the stress-strain tissue data. The model was separately applied to two datasets: the ligamentous sheath data, and central core data, for the eight cetacean pieces and the five regions of the killer whale fin.

Objective #3:
With additional tag deployments in the last year, we have updated our examination of tag attachment durations and applied more rigorous modeling. To assess the factors which might have influenced tag attachment duration for LIMPET tags, we examined a subset of the data for tags deployed between April 2008 and September 2014 from the three odontocete species with the largest sample sizes: short-finned pilot whales, false killer whales, and killer whales. We excluded tags documented to have cracked on impact (n = 4) and tags which were deployed below the dorsal fin (n=6), leaving 136 tags from these three species. Using a generalized linear model we assessed vertical and horizontal placement on the fin, how flush the tag was deployed (*e.g.* whether the leading edge of the tag lifted up into the flow of water), the number of darts which were successfully embedded into the fin, the number of darts which penetrated out the opposite side of the fin, the tag shape version, and the titanium dart style. As described in previous annual reports, the LIMPET tag has undergone a few shape changes, due to innovations to improve impact resistance and in response to lessons learned from our hydrodynamics work. The influence of titanium attachment dart types was assessed by comparing two types: our previous version of the titanium dart (custom-built at ASLC) and the recent dart with improved strength (as detailed in the previous annual report) which was available from Wildlife Computers beginning in November 2012. Six tags were noted in the field to have been removed from the fin of the tagged whale during the time of observation within weeks of tagging, likely due to conspecific interactions. These tags were not excluded from the analysis, as this same interaction could have occurred at any time for other deployments when observers were not there to document the removal. We assessed many of the same factors within species as well, although some variables had to be dropped from the model due to insufficient sample sizes. We were able to assess a few additional variables within species: for pilot whales we added region (Cape Hatteras, Atlantic Ocean vs. Hawaii,

Pacific Ocean), age class and sex. For killer whales we added sex and ecotype (fish-eating, mammal-eating, and offshore), and for false killer whales we added sex.

Objective #4:
Although no funds in FY14 were directed at field work for this objective, progress was made on the following topics: 1: Obtaining additional follow up photos from collaborators and through projects funded for other purposes. 2: Matching of photos for species that had been satellite tagged for addition to our long-term photo-ID catalogs. 3: Association analyses to identify stable groupings ("clusters") and assessments of re-sightings and reproduction of tagged individuals. 4: quantitative estimation of survival of tagged and non-tagged individuals using a capture-recapture framework, for the two species with the largest samples sizes of tags deployed and photo-identifications (false killer whales and short-finned pilot whales).

Field projects funded by other sources were undertaken off Hawaiʻi Island in Oct/Nov 2013 and July 2014, and photos were obtained from collaborators off Oʻahu and Hawaiʻi Island. All photographs obtained through Aug. 2014 of 4 of the 8 tagged species with photo-ID catalogs (false killer whales, pygmy killer whales, Cuvier's beaked whales, Blainville's beaked whales) have been matched to the catalogs to identify previously tagged individuals. New photos of short-finned pilot whales from Hawaiʻi Island have been added to the catalog, prioritizing groups known to contain tagged individuals.

RESULTS

Objective 1:
In our previous annual reports we detailed the findings from both CFD and water tunnel tests with physical models. Both methods demonstrated the previously unrecognized importance of lift in the total hydrodynamic force acting on the tag. These extensive results were summarized at the recent 5th International Biologging Conference in Strasbourg, France (September 2014) and are included in a manuscript currently in preparation, but one key take home message is that the major factor limiting tag attachment durations is not likely static hydrodynamic force, but rather it is the sudden dynamic force of impacts with other whales or objects such as the ocean bottom. Our initial view of how tag detachment occurred is illustrated in Fig. 1. Although we cannot measure it directly, our visual observations of tags on whales over time and our simulations in the lab suggest that the barb retention force of the tissue decreases over time due to tissue remodeling in the wound. We originally hypothesized that the tag would eventually detach when this barb retention force was overcome by the hydrodynamic pulling force exerted on the tag, primarily by water flow. Our original hypothesis was that this hydrodynamic pulling force would increase over time as the tag migrated away from surface of the dorsal fin and towards the outer edge of the boundary layer. However, the CFD and water tunnel testing showed that in fact the overall hydrodynamic force actually decreases at first, because as the tag starts to pull away from the fin, water flow between the tag and the fin results in less lift force. But once the gap is more than 0.12 times the height of the tag (2.5 to 3 mm), the drag force will dominate and the total hydrodynamic force does increase as the tag pulls further away from the fin. Hence the u-shaped curve for hydrodynamic force in Fig. 2. However, our measurements of the retention force of implanted darts in carcass tissue demonstrate that this force is nearly an order of magnitude greater than the total hydrodynamic pulling force, even at very high swimming speeds. These results, along with our observations of large variability in tag attachment duration that is seemingly unrelated to any of the explanatory variables we have examined, have led us to suggest that in the vast majority of cases

it is a sudden impact force that leads to tag detachment, not the slow but eventual intersection of the retention and steady hydrodynamic forces (Fig. 2).

Objective #2:

The collagen composition (by %) in the ligamentous sheath of the dorsal fins was consistently high in all species, ranging from a high of 96% in the pilot whales to a low of 75% in Blainville's beaked whales (Fig. 3). Variability in collagen % between species was greater for the central core tissue, with pilot whales again having the highest percentage of collagen (81%), and the Blainville's beaked whale the lowest at 42%. For the different regions in the killer whale dorsal fin, the central and anterior areas had the highest percent collagen for ligamentous sheath tissue, while for the central core the dorsal and posterior areas were the highest, with their collagen content being very similar to the ligamentous sheath (Fig. 4).

The stress/strain curves for ligamentous sheath and central core tissue of the eight cetacean species all exhibited the non-linearity associated with collagen-bearing viscoelastic tissues. The ligamentous sheath generally displayed a greater yield point for a particular strain value than did the central core. For the central core the slopes in the elastic region for all species were more variable and generally lower than those observed for the ligamentous sheath, as were yield points. High yield points were observed for Blainville's beaked and Bryde's whales (Fig. 5), and although harbor porpoise was the lowest, the killer whale tissue was observed relatively low.

Surprisingly, the material properties results did not correlate with the collagen composition values. In general, the yield points for the ligamentous sheath tissues were unrelated to percent collagen. The two species with the highest collagen content in their ligamentous sheath, pilot whale and killer whale, had the two lowest yield points, and Blainville's beaked whale, with one of the highest yield points, had the lowest collagen percentages. A somewhat similar pattern was seen in a comparison of central core yield points and percent collagen, with the highest yield point in Blainville's beaked whale, which had the lowest percent collagen, and relatively lower yield points for pilot, false killer, and killer whale which had relatively higher percent collagen values.

Objective #3:

For the 136 tags on short-finned pilot whales, false killer whales and killer whales chosen for the analysis of factors affecting tag attachment duration, the overall median transmission duration was 35.2 days (range = 0 - 228.3 d, n= 136). In the GLM, the only factor that had a significant impact on tag attachment duration was whether both darts had hit the fin and successfully implanted. The tags that hit the margins of the fin resulting in only one of the two darts being implanted into the fin stayed attached for significantly less time. While there were trends in several other factors, none of them were significant in the model. The new design of attachment darts resulted in a median transmission duration 9.1 days longer than the older version of darts (41.9 d vs 32.0 d), but this was not significant (GLM, $p = 0.067$). Tags deployed with the leading edge up (lifted into the flow of water; Fig. 6) had a median transmission duration of 16.9 days, much shorter than the overall median, but this was not significant in the GLM. Dart penetration through the opposite side of the fin had no effect on tag attachment duration.

Despite a large number of variables that may influence tag attachment duration, tags deployed with their leading edge lifted up into the flow of water had the shortest attachment durations, consistent with results from the hydrodynamic studies that demonstrated large increases in drag at positive pitch

angles. Of the tags that had transmission durations longer than 50 days, all of them had two darts implanted into the fin at time of attachment, and they were all flush, i.e. none of them had the leading edge of the tag lifted into the water flow, suggesting that these factors may be important for keeping tags attached for longer durations (Fig. 6).

Within short-finned pilot whales only (n=67), the only significant factor in attachment duration was age class, with sub-adults having a significantly longer attachment duration than adults (generalized linear model, p=0.047; Fig. 7). Tags deployed off Cape Hatteras in the Atlantic (n = 14) had a median transmission duration of 57.7 days compared to 36.7 from Hawaii (n=53), but again, the results were not significant. Previous observations have led us to hypothesize that tags deployed in warmer waters tend to stayed attached for shorter periods on the same species. The between-region comparison of short-finned pilot whales seems remarkable, but the lack of significance despite a reasonable sample size illustrates the problems we encounter in these analyses, primarily due to very large variability in attachment duration. Within killer whales (n=38), male killer whales had significantly shorter attachment durations (generalized linear model, p=0.018) than females, although the difference was only 5 days. Mammal eating killer whales had shorter transmission durations (median = 27.3, range = 2.2 - 94.3, n=24) than fish eating (median = 38.3, range = 2.7 - 160, n=11) and offshore ecotypes (median = 56.7, range = 8.3 - 98.8, n=4), although the difference was not significant (p=0.058; Fig. 8). These results tend to confirm our hypothesis that the primary factor affecting tag attachment is whether, or when, the tag is subjected to a sudden but uncommon impact force.

IMPACT/APPLICATIONS

Understanding the potential for impacts of naval activities on protected species of marine mammals and mitigating such impacts requires information on movements and habitat use. The development of better tag technologies and deployment techniques will make a significant contribution to the ability of researchers to track movements, monitor behavior, and determine distribution of species of interest.

TRANSITIONS

The improved versions of the LIMPET satellite tag and attachment system that were developed during this project are now commercially available from Wildlife Computers (Seattle, WA), and LIMPET tags are being acquired by and deployed by multiple organizations (e.g. Southwest Fisheries Science Center, Cascadia Research Collective, Woods Hole Oceanographic Institution, among many others) in other projects funded by the US Navy to monitor marine mammals on Navy ranges and elsewhere.

RELATED PROJECTS

The National Marine Fisheries Service Pacific Islands Fisheries Science Center is supporting research on false killer whale movements in Hawaiian waters (Baird et al. 2010; 2012), and the Naval Postgraduate School (with funding from N45) is supporting tagging studies of a variety of species. Tag and deployment developments from this work are being incorporated into these ongoing studies. For example, see: www.cascadiaresearch.org/hawaii/beakedwhales.htm
www.cascadiaresearch.org/hawaii/falsekillerwhale.htm
www.cascadiaresearch.org/SCORE/SCOREMain.htm

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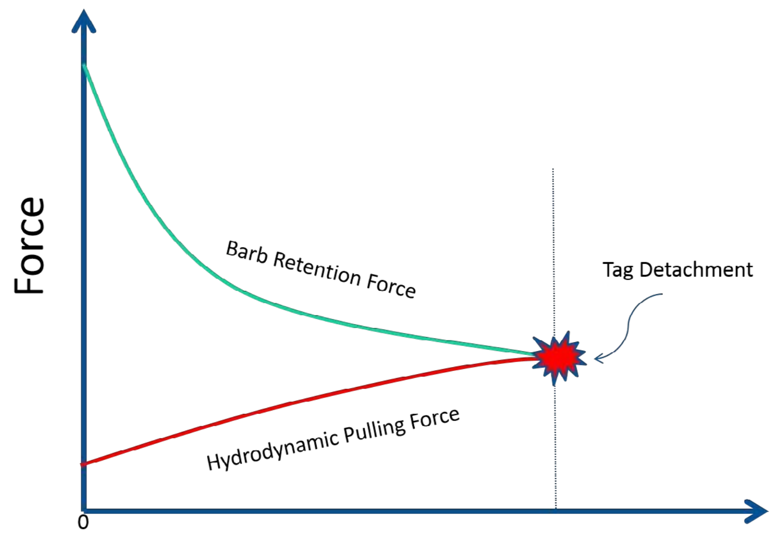

Fig. 1. Illustration of our initial view of how the barb retention force of the tissue changes over time due to tissue remodeling in the wound, and how the hydrodynamic pulling force was thought to increase over time as the tag migrated away from fin surface and towards the outer edge of the boundary layer. Under perfect conditions, our original hypothesis was that tag detachment occurs when this hydrodynamic pulling force exceeds the barb retention force of the tissue.

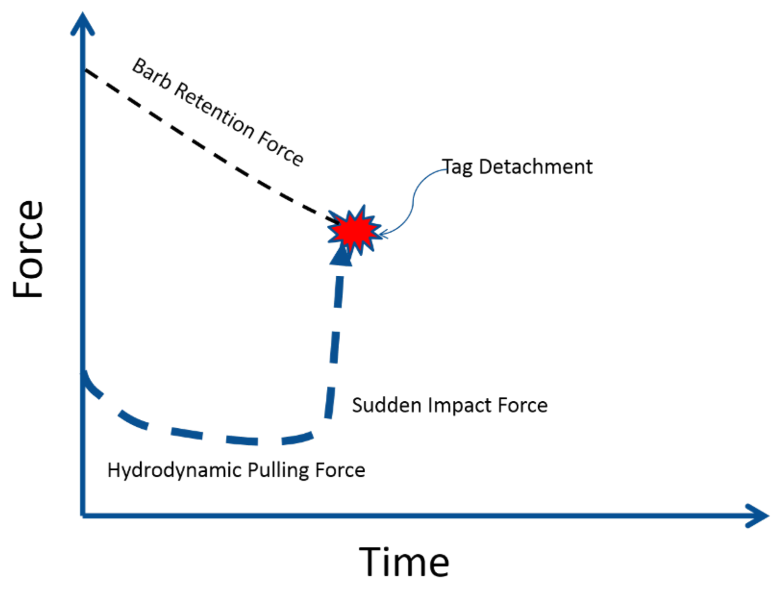

Fig. 2. Illustration of our revised view of the tag detachment process. CFD and water tunnel testing demonstrated that the overall hydrodynamic force decreases at first, because as the tag starts to pull away from the fin, water flow between the tag and the fin results in less lift force. However, long before the retention force can be overcome by static hydrodynamic pulling force, most tags are likely detached by a sudden impact force that is an order of magnitude higher.

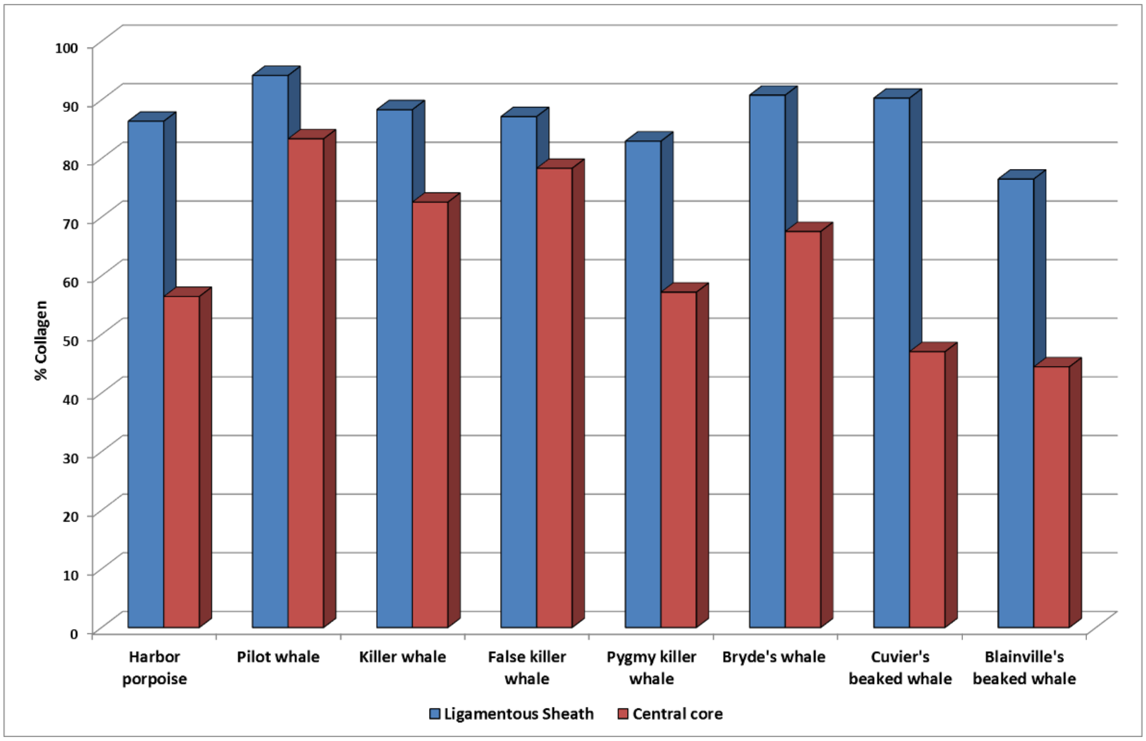

Fig. 3. Percent collagen in the ligamentous sheath and central core of the dorsal fins of eight cetacean species.

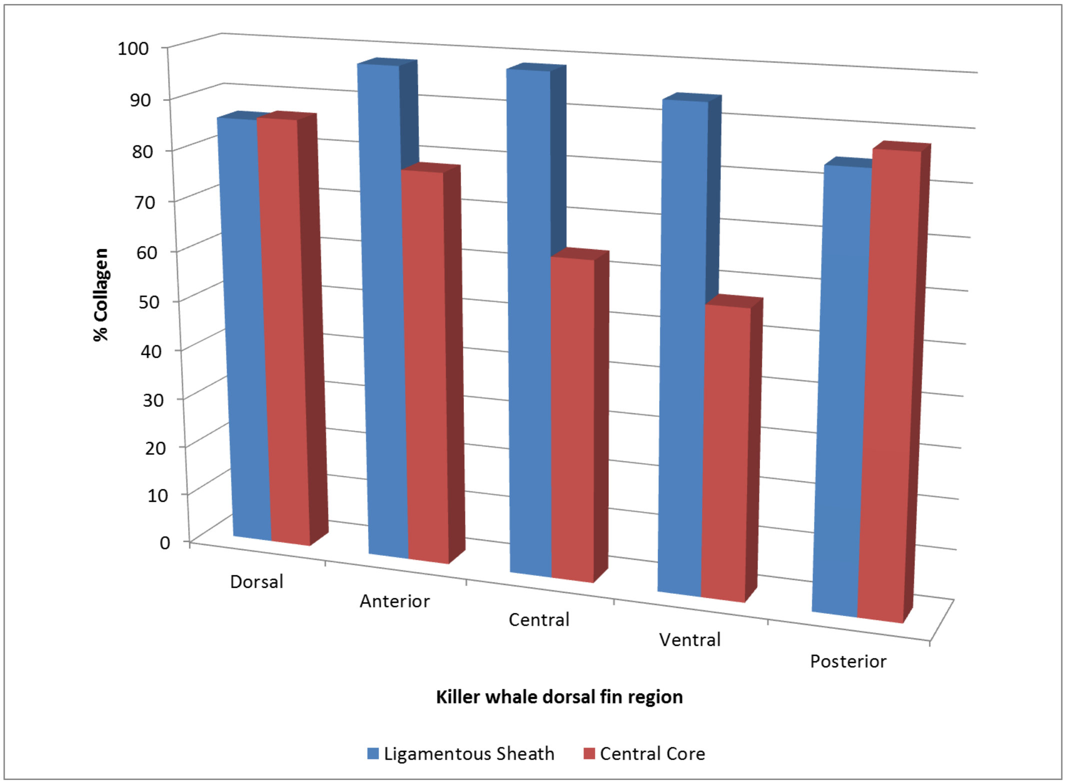

Fig. 4. Collagen % in ligamentous sheath & central core for five regions of the killer whale dorsal fin.

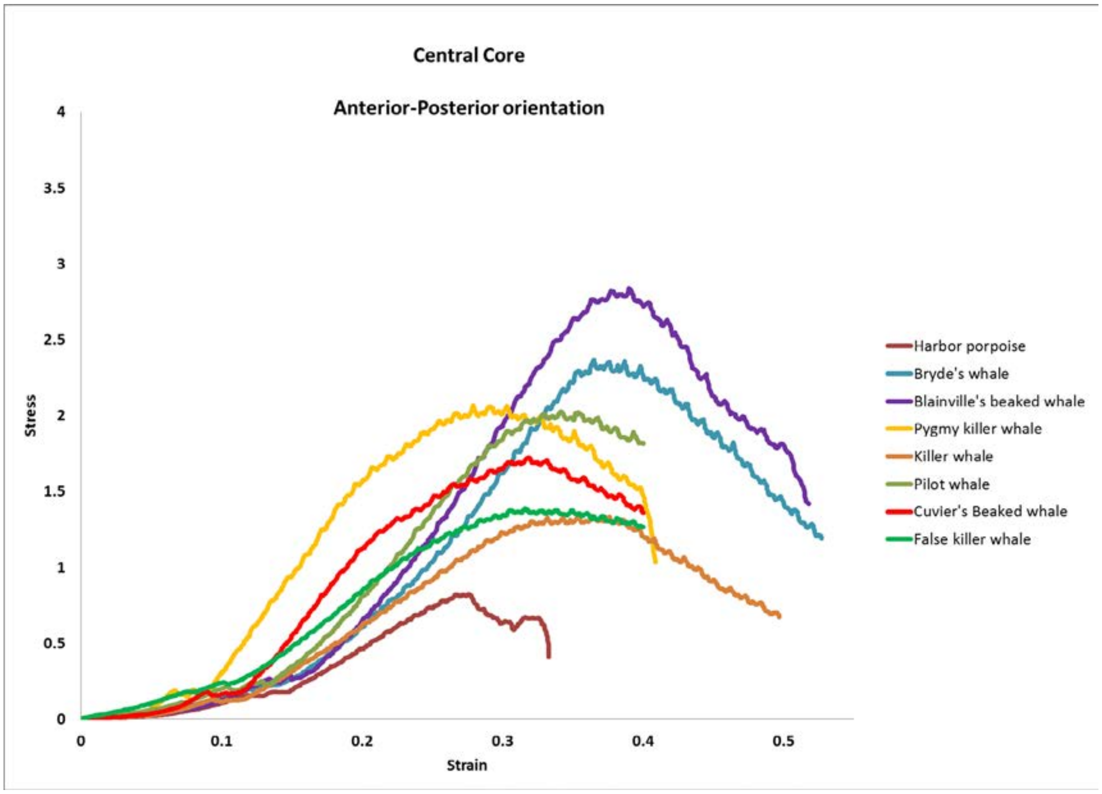

Fig. 5. Stress/strain curves for the central core in the dorsal fin of for eight cetacean species.

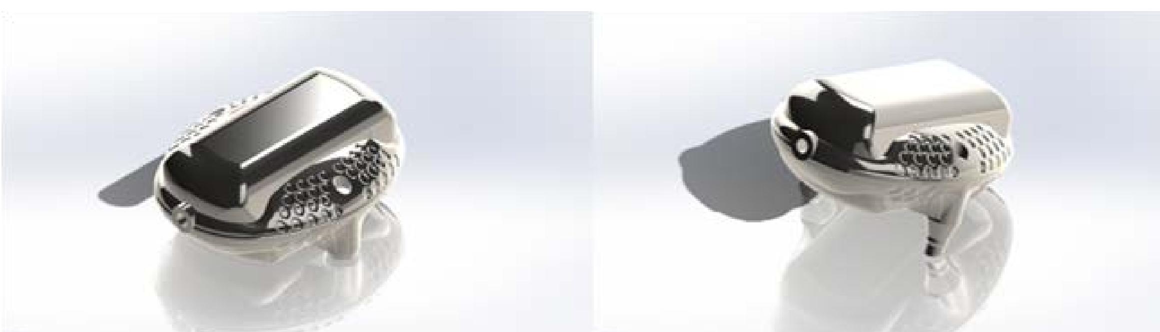

Fig. 6. Illustration of a flush tag, with pitch angle of 0° (left), and a tag with the leading edge lifted into the flow of water, pitch angle = 20° (right) Water flow direction: lower left to upper right. A pitch angle of 20° caused a significant increase in drag force in both CFD and water tunnel studies, while all of the tags deployed on whales that stayed attached for more than 50 days were flush upon attachment.

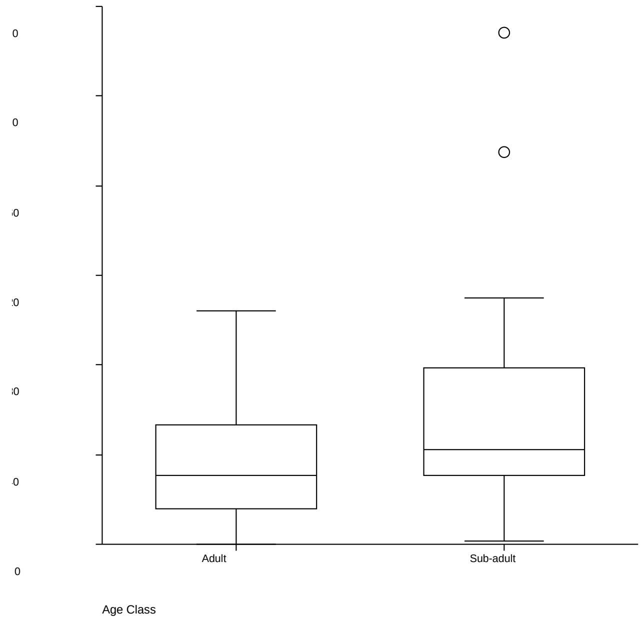

Fig. 7. Differences in transmission duration (presumed equal to attachment duration) between age classes of short-finned pilot whales.

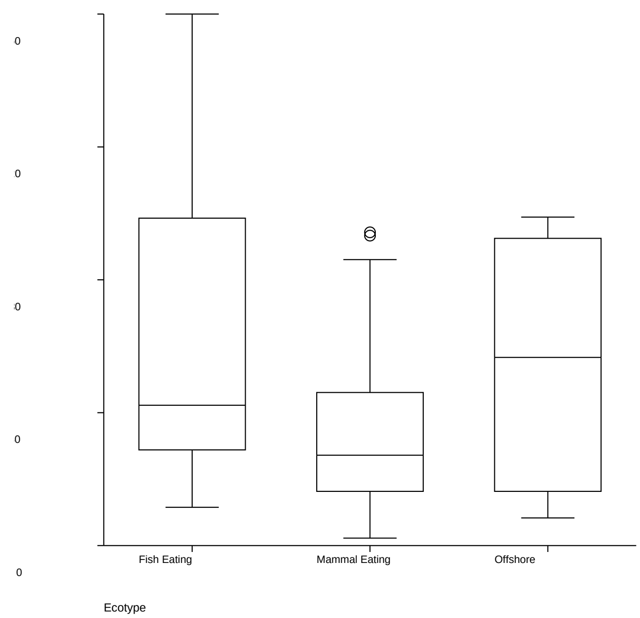

Fig. 8. Transmission (~ = attachment) duration by ecotype for killer whales. While mammal eating killer whales had the shortest transmission duration, the difference was not significant.